MODEL: (3PA31) Intraperitoneally-Implanted Adriamycin Resistant P388 Leukemia

Origin of Tumor Line: P388 leukemia was chemically induced in 1955 in a DBA/2 mouse by painting the skin with 3-methylcholanthrene.[1] The adriamycin-resistant P388 leukemia was established at Southern Research Institute in 1973 by treating a P388 leukemic B6D2F1 mouse i.p. with NSC 123127 at 1.5 mg/kg daily, days 4 - 12; treatment was at 6 mg/kg, day 4 only at the third passage. Treatment to maintain resistance is 4 mg/kg, i.p. day 2 only. The 3PA31 test system is 4 logs less sensitive to NSC 123127 than the sensitive parent P388 leukemia when both tumors are treated in vivo with a single MTD in parallel groups of mice.

Summary of Test Procedures: 1×10^6 cells in ascitic fluid are implanted i.p. in CD2F1 mice. Test agent treatment is as instructed. Results are expressed as a percentage of control survival time. A sensitive P388 leukemia (3PS31) control is to be run in parallel.

ANIMALS: (refer to Protocol 8)

Propagation: CD2F1 mice.
Testing: CD2F1 mice.
Weight: Mice should be within a 3 gm weight range with a minimum weight of 18 gm for males and 17 gm for females.
Sex: One sex is used for all test and control animals in one experiment.
Source: One source, if feasible, for all animals in one experiment. Exceptions to be noted as comments.

EXPERIMENT SIZE: (refer to Protocol 9)

General Testing: Ten animals per test group.
Control Groups: Number of control animals varies according to number of test groups.
Titrations: Each control is to include titrations of 1×10^7 to 1×10^3 cells, inclusive, with ten animals per level.

TUMOR TRANSFER: (refer to Protocols 2, 5, and 6)

Propagation:

Suspension: Prepare a suspension of diluted ascitic fluid so that 0.1 ml portion contains 1×10^6 cells.
Time: Day 7
Site: Implant i.p. 0.1 ml of suspension containing 1×10^6 cells.
Treatment to maintain resistance: NSC 123127, 4 mg/kg/inj., Day 2 only.

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[1]American Journal of Pathology, 33 No. 3. p 603, 1957.

Testing:

Suspension: Prepare a suspension of diluted ascitic fluid so that 0.1 ml portion contains 1 x 10^6 cells.

Time: Day 7

Site: Implant i.p. 0.1 ml of suspension containing 1 x 10^6 cells.

TESTING SCHEDULE: (refer to Protocols 3 and 4)

Day 0: Implant tumor. Run bacterial cultures (refer to Protocol 7). Prepare materials. Test positive and negative control compounds in every experiment. Record deaths daily.

Day 1: Check cultures. Discard experiment if contaminated. Record deaths daily. Randomize and weigh animals. Treat as instructed.

Day 2: Recheck cultures. Discard experiment if contaminated.

Day 5: Weigh animals and record. Toxicity day.

Day 7: Control early-death day.

Day 18: Control no-take day.

Day 20: If there are no survivors except those treated with positive control compound, end and evaluate experiment.

Day 30: End and evaluate experiment.

QUALITY CONTROL: (refer to Protocol 7)

Schedule the positive control compound (NSC 19893* at a dose of 25 and 15 mg/kg/injection) in every experiment, the regimen for which is i.p. QD 1-5 for Synthetics. The lower T/C limit for the positive control is 135%. Schedule the negative control compounds in every experiment; NSC 123127** at doses of 15, 10, 6.7, 4.5 mg/kg/inj. day 1 only and NSC 67574** at doses of 3, 2.0, 1.5, 1.0 mg/kg/inj. days 1, 5 and 9. The acceptable untreated control median survival time is 9 - 14 days.

EVALUATION: (refer to Protocol 11)

The parameter measured is median survival time. Compute mean animal body weights for Day 1 and Day 5, compute T/C for all test groups with >65% survivors on Day 5. A T/C value of <86% indicates toxicity. An excessive body weight change difference (test minus control) may also be used in evaluating toxicity.

CRITERIA FOR ACTIVITY:

To be established.

REPORTING OF DATA:

On the final day of testing, prepare final control and test reports. A comment must be provided to cross reference both the resistant (3PA31) and sensitive (3PS31) control numbers.

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*Positive control compound: NSC 19893 is 5-FU. CAS RN is 51-21-8.

**Negative control compounds: NSC 123127 is Adriamycin. CAS RN is 25316-40-9. NSC 67574 is Vincristine. CAS RN is 57-22-7.

Assign a Test Status Code (TSC) of 33 to any test group the screener considers to be invalid for any reason.

A comment must be provided stating the reason for a TSC of 33, when a nonstandard dose is administered (whether due to a solubility problem or special request) and for poor suspensions.